SN 10/656,358
Docket No. S-100,543
In Response to Office Action dated 10/26/2005

REMARKS

Claims 1-10 are pending. Claim 1 has been amended. No new matter has been introduced.

A Declaration of Donna L. Robinson is submitted under 37 CFR 1.132 herewith.

1. Formalities Objection:

The Office objected to the specification on the grounds that it contains color drawings.

A Petition under 37 CFR 1.84(a)(2) was submitted, and the corresponding fee under 37 CFR 1.17(h) was paid, at the time the application was filed on September 4, 2003. A copy of the Petition as filed is included herewith. If the Petition is not granted, applicants will submit replacement black and white drawings.

2. Rejections under 35 USC 103:

Claims 1-10 were rejected under 35 USC 103(a) as being unpatentable over Roche Diagnostics GmbH, "GC-Rich PCR System", July 2001 ("Roche"), in view of Landre et al., 1995, and Tabor et al., 1997.

The Office noted that while Roche *"do not specifically teach extensions at 75, the claims are drawn to temperatures of 'about 75'"* (i.e., step "d" of claim 1). The Office concluded that *"the extension temperatures of Roche clearly suggest the claimed 'about 75' "*.

Claim 1 has been amended to omit the term "about" in relation to the range of 75-78 °C recited in step "d".

The Declaration of Donna L. Robinson (the "Robinson Declaration") is submitted as evidence traversing the rejection and as evidence in support of the non-obviousness of the claimed invention generally.

In her Declaration, Robinson describes the development of the claimed invention (paragraphs 1-5 and Exhibit A) and the unexpected results she and others at the Los Alamos National Laboratory ("LANL") obtained using the claimed methods (paragraphs 6-7). She further provides evidence that the claimed methods were very well received and adopted by her LANL colleagues, and that the invention became a critical element in closing the sequencing gaps that known methods had failed to close (paragraph 6 and Exhibit B).

It is clear from the Robinson Declaration that LANL's "R&D Team" tried numerous approaches to sequencing difficult regions of DNA characterized by high G-C content and/or CCT repeat sequences. One of the approaches attempted by Robinson's colleagues was the procedure described in the cited Roche reference. However, none of the approaches were effective at closing the sequencing gaps caused by these difficult regions, including the approach described in Roche.

Exhibit B to the Robinson Declaration contains copies of several e-mail communications attesting to the successful use and significance of the invention in the context of LANL's sequencing efforts. In these e-mail communications to Robinson, her colleagues express thanks, congratulations and encouragement to Robinson for the application of her "GC Buster" method to LANL's difficult sequencing problems. These communications indicate that the method was very well received by her colleagues and was critical to finishing sequencing gaps due to high G-C and CCT repeat content areas, becoming the "major method to finish gaps" and the method producing the "best results".

In regards to the Roche reference, specifically, Robinson states that there is little similarity between the procedure outlined in Roche and the methods of the invention

(paragraphs 8-9). Although the Office indicates that Roche teaches examples of sequencing using high Td primers, Roche contains no indication of the use of high Td primers. It would be an exercise in hindsight reasoning to somehow infer that this critical deficiency is present in Roche (which the office has not done) – the reference is completely silent on this point. Moreover, LANL's R&D Team applied the Roche method, without success, and apparently did not think of modifying the conditions of Roche or using high Td primers. Among the many people involved in LANL's sequencing efforts, only Donna Robinson conceived of the combined use of high temperature annealing and extension conditions, further combined with high Td primers.

Roche describes a set of conditions for PCR amplification (not cycle sequencing) that diverge considerably from the steps of the claimed invention. First, in regards to the method of claim 1, the invention teaches the use of high Td primers, whereas Roche does not even suggest the use of high Td primers (essentially teaching the use of standard primers). The invention teaches high temperature annealing, between 65 and 67 °C, whereas Roche discloses annealing at a temperature of between 45 and 65 °C (a much wider and cooler range). Finally, the invention teaches a high temperature extension step, between 75 and 78 °C, for a time period of 3-4 minutes, whereas Roche discloses extension at 68 or 72 °C for a considerably shorter time period. Robinson explains why these differences are significant, and provides a potential explanation for why her LANL colleagues were unable to apply the procedure of Roche successfully.

Robinson also explains certain results presented in the application, wherein the high temperature cycling conditions employed in the claimed methods, alone, proved substantially inferior relative to the use of these conditions in combination with high Td primers, thereby underscoring the importance of the use of high Td primers in combination with the other conditions employed in the method. Robinson further explains that the quality of the sequence information generated with the methods of the invention was unexpectedly "very high" over exceptionally long read-lengths.

Accordingly, the invention as a whole is considerably different from the teachings of Roche.

Neither the Landre et al or Tabor et al references teach or suggest the claimed invention either, or supply the missing deficiency of high Td primers. The Office states that Landre et al. indicate that the optimal temperature for DNA synthesis using Taq polymerase is 75-80, and teach that the use of higher temperatures for annealing and extension increases specificity, yield and sensitivity of the amplification. However, Landre et al is a 1995 publication that specifically addresses enhancement of amplification using PCR, not the conditions under which cycle sequencing of DNA having high G-C or CCT content should be carried out. In view of the huge volume of published literature relating specifically to cycle sequencing of DNA that had piled-up at the time the invention was made, it is unlikely that those of skill in the art would have considered Landre et al significant or would have combined its teachings with Roche, especially if in conflict with the cycle sequencing body of literature. Indeed, at the time the invention was made, conventional cycle sequencing conditions were well established and in conflict with the conditions suggested by Landre et al for PCR. As indicated in Exhibit A to the Robinson Declaration, conventional cycle sequencing conditions included primer annealing at 50 °C for only 5 seconds, followed by extension at 60 °C. This combination of annealing and extension temperatures was standard in the automated cycle sequencing field at the time the invention was made – see, for example, pages 3-27 and 3-28 of reference number 39 in applicant's previously-submitted Information Disclosure Statement, Applied Biosystems' "*Automated DNA Sequencing Guide*". The conditions indicated include annealing at 50 °C for 5 seconds and extension at 60 °C for 4 minutes. Thus, despite Landre et al's indication that Taq runs optimally at 75-80 °C, and that higher temperatures for annealing and extensions could be used in PCR amplification reactions, the automated sequencing field did not adopt such conditions.

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The Robinson Declaration provides ample evidence of unexpected results and the adoption and use of the invention to solve sequencing problems that LANL's Finishing Team and incorporated R&D Team could not solve despite the application of various techniques, methods and tricks for sequencing G-C rich DNA or DNA containing CTT repeats. Thus, the Robinson Declaration provides strong evidence of non-obviousness. As Robinson's evidence shows, the invention's success is dependent on a delicate balancing of a variety of conditions. Neither Roche, Landre or Tabor, alone or in combination, put all the pieces together, or provided one of skill in the art with guidance in the direction of the claimed invention. The problem of sequencing through regions of high G-C content had been known for many years and had generated many publications, commercial products and methodologies purporting to solve the problem, over a long period of time (see, for example, the references presented in the Information Disclosure of record in this application). As Robinson states, these methods and products were available and used by a team of researchers at LANL without success. Finally, the Robinson Declaration establishes that, at least at LANL, the methods of the invention satisfied a long felt need and provided success where other methods had failed.

Applicants kindly request reconsideration and withdrawal of the rejection in view of the foregoing amendments to the claims, the evidence provided by Donna Robinson, and these remarks.

Respectfully submitted,

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